Nonsystemic Bunt Fungi—*Tilletia indica* and *T. horrida*: A Review of History, Systematics, and Biology*

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Key Words

Karnal bunt, *Neovossia*, rice kernel smut, *Tilletia walkeri*, Tilletiales

Abstract

The genus *Tilletia* is a group of smut fungi that infects grasses either systemically or locally. Basic differences exist between the systemically infecting species, such as the common and dwarf bunt fungi, and locally infecting species. *Tilletia indica*, which causes Karnal bunt of wheat, and *Tilletia horrida*, which causes rice kernel smut, are two examples of locally infecting species on economically important crops. However, even species on noncultivated hosts can become important when occurring as contaminants in export grain and seed shipments. In this review, we focus on *T. indica* and the morphologically similar but distantly related *T. horrida*, considering history, systematics, and biology. In addition, the controversial generic placement and evolutionary relationships of these two species are discussed in light of recent molecular studies.

INTRODUCTION

Dikaryon: a cell with two genetically distinct, haploid nuclei

Smut fungi belong to class Ustilaginomycetes, phylum Basidiomycota. The class name is derived from "ustulatus," meaning burned, in reference to the blackened appearance of infected plants (22). Cereal-infecting species of *Tilletia* that produce teliospores in the ovaries of their hosts are commonly called bunt fungi, also thought to be a derivation of the word burned (21, 38). Wheat bunts are considered among the most important fungal plant pathogens and were the focus of early plant pathological treatises, such as that by M. Mathieu du Tillet (129). Tillet (129) showed that the cause of wheat bunt was from seed contaminated by the "greasy and blackish powder" contained within afflicted kernels before there was an understanding of the role of fungi in plant disease. Tillet's contributions are reflected in the generic name, *Tilletia* Tul. & C. Tul., established in 1847 (133).

Tillet worked with "stinking smut" caused by *Tilletia caries* (DC.) Tul. [= *T. tritici* (Bjerk.) Wint.], the common bunt pathogen that infects wheat at the seedling stage. Seedling infection, where the fungus grows systemically and sporulates remote from the site of initial infection, became established as the mode by which all smut fungi were presumed to infect and colonize their hosts. When seedling inoculation methods proved ineffective for corn smut [*Ustilago maydis* (DC) Corda], a second mode of infection, "local infection," was elucidated. In local infections, growth and sporulation typically occurs near the site of infection (42).

Karnal bunt, caused by the locally infecting *Tilletia indica* Mitra [≡*Neovossia indica* (Mitra) Mundkur], is a disease with limited geographic distribution and minor yield impact under most environmental conditions (10, 15, 45, 48, 107, 112). Karnal bunt was discovered in North America in Mexico in 1972 (36), and in 1983 the United States and approximately 70 other countries placed quarantines on wheat from countries where Karnal bunt was known to occur. There are few publications on the biological differences between locally and systemically infecting bunt fungi. In this review we focus on *T. indica* and the morphologically similar but distantly related rice kernel smut pathogen *Tilletia horrida* Takah., considering their history, systematics, and biology.

HISTORY

The Genus *Tilletia*

Many locally infecting bunt fungi have been classified in both *Tilletia* and *Neovossia* Körn. The type species of *Tilletia*, *T. caries*, infects wheat systemically and has pale yellow to reddish-brown reticulate teliospores (**Figure 1**) interspersed with hyaline thick-walled sterile cells (38). Infected wheat has a fetid smell due to the production of trimethylamine (90). The teliospores germinate to form a terminal whorl of 4–16 primary basidiospores, which conjugate almost immediately to form a dikaryon (50). The genus *Neovossia* was created in 1879 for *Vossia moliniae* Thüm., a species with pitted elongated teliospores

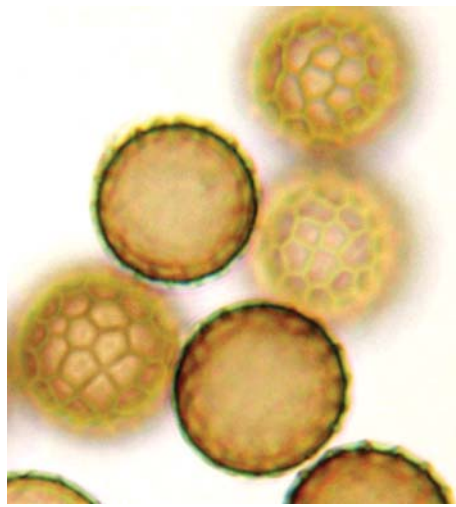

Figure 1

Teliospores of *T. caries*, the type species of the genus *Tilletia*, showing reticulate ornamentation. Diameter of teliospores ranges from 16 to 22 μm.

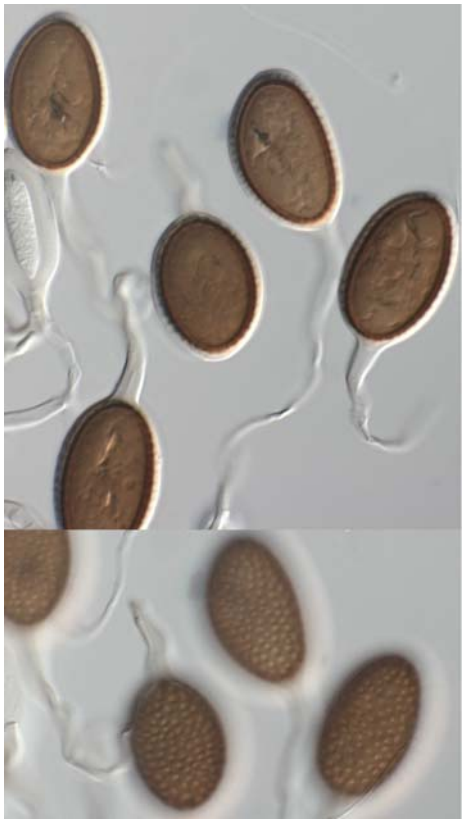

Figure 2

Teliospores of *Neovossia moliniae* in median view showing the long hyaline appendage (*top*) and in surface view showing the pitted exospore ornamentation (*bottom*). Teliospores are typically 14 to 18 μm wide by 18 to 30 μm long.

(**Figure 2**) described from *Molinia caerulea* (L.) Moench (70). Brefeld (19) described and illustrated the germination characteristics of *N. moliniae* (Thüm.) Körn., along with a new species, *N. barclayana* Bref. from *Pennisetum triflorum* Nees. *Neovossia moliniae* and *N. barclayana* are characterized by formation of a large number of primary basidiospores that do not conjugate, with *N. barclayana* forming globose tuberculate teliospores. Approximately 12 additional species have been described as or transferred to *Neovossia* (41), including *T. indica* [as *Neovossia indica* (Mitra) Mundkur] and *T. horrida* [as *Neovossia horrida* (Takah.) Padwick & A. Khan]. The distinction between *Neovossia* and *Tilletia* has been controversial.

Tullis & Johnson (134), in a study of *T. horrida* and *N. barclayana*, emended the description of *Neovossia* to include species producing teliospores with a hyaline sheath, fragmentary appendages that may or may not persist after maturity, large numbers of nonconjugating primary basidiospores, and two types of sporidia. They also erroneously reported that *Tilletia* species produce only one type of sporidium. This concept has been used by others (114, 143) to support the placement of *T. horrida* and *T. indica* in *Neovossia*. Goates & Hoffmann (53, 54) clearly demonstrated that the formation of two types of sporidia is typical for species of *Tilletia*. In addition, differences in number and fusion of primary basidiospores have been used to support the placement of *T. horrida* and *T. indica* in *Neovossia* (68, 69, 71, 114, 118, 143).

Vánky (136) considered *Neovossia* to include only *N. moliniae*. He listed localized infection, long hyaline appendages on the teliospores, and the absence of sterile cells and trimethylamine smell as important characteristics of *Neovossia*. Recent molecular work (25) indicates that there is no phylogenetic support for maintaining *Neovossia* as distinct from *Tilletia*. Although this study supports four main lineages within the genus *Tilletia* sensu Durán & Fischer (38), none corresponds exclusively to taxa with the morphological characters used by Vánky (136) to define *Neovossia*. In addition, locally infecting taxa with large numbers of nonconjugating basidiospores do not form a single phylogenetic entity, indicating that these characters are not informative at the generic level. Castlebury et al. (25) included all species that have been segregated into other genera, including *Neovossia*, in the genus *Tilletia*.

Sporidia(-ium): secondary spores formed by Tilletiales; in a broad sense used for any spore in the life cycle of smuts other than a teliospore

Karnal Bunt—*Tilletia indica*

Tilletia indica was first described in Karnal, Punjab, India in 1931 (80). *Tilletia indica* causes partial conversion of individual

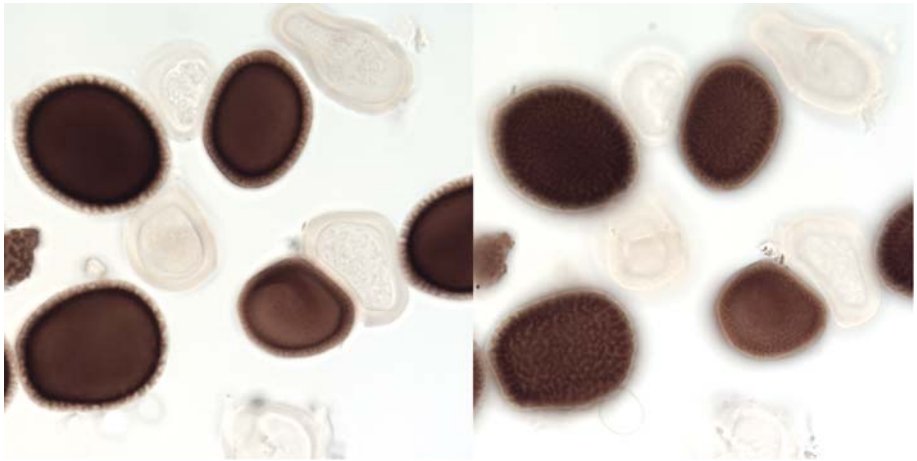

Figure 3

Dark brown teliospores and hyaline to yellowish sterile cells of *T. indica*, cause of Karnal bunt of wheat, in median (*left*) and surface (*right*) views, showing finely tuberculate to fused and cerebriform exospore ornamentation. Teliospores can range from 26 to 54 μm in diameter.

kernels into sori filled with teliospores and sterile cells (**Figure 3**), resulting in losses in both yield and quality. Kernels have varying degrees of disease ranging from small point infections at the embryo end to kernels that are almost completely converted into a dark mass of teliospores (**Figure 4**). Mundkur (82) stated that *T. indica* "probably belongs to the genus *Neovossia*" based on the large number of nonconjugating basidiospores formed by the fungus and provided the new combination *N. indica*. In 1940, Mundkur (83) noted the fetid smell associated with the spore mass and the difference from *T. caries* (as *T. tritici*) in the partial destruction of the kernels. He later provided evidence that *T. indica* was not a systemic disease and generally had no more than five or six infected kernels per head (84).

Figure 4

Karnal bunt of wheat showing infections ranging from small point infections to kernels almost completely converted into teliospores. Hollowed-out kernels result when teliospores filling the kernel are dislodged during threshing.

Until the early 1970s the distribution of *T. indica* was restricted to Asia and included most of northwestern India (85, 107). In 1972, it was reported in Sonora, Mexico and by 1983, it was being intercepted in railroad cars entering the United States from Mexico (18). In 1996, *T. indica* was first reported in Arizona (145), resulting in the U.S. Department of Agriculture declaring an "extraordinary emergency" and a national survey for the presence of *T. indica*. Currently, the distribution of *T. indica* includes small areas in California, Arizona, and Texas in the United States; Afghanistan, India, Iran, Iraq, Mexico, Nepal, Syria; and the Northern Cape Province of South Africa (104, 107).

The incidence of Karnal bunt varies considerably from year to year due to its

dependence on favorable weather during heading (10, 44, 48, 107, 112, 113). The disease has historically caused minor overall yield and quality losses in countries where it occurs (20, 107, 112). Significant yield or quality losses are typically localized, occurring in highly susceptible cultivars grown in fields with high inoculum density during seasons with unusually favorable weather. Within Mexico and the United States, most economic losses have come from the effects of quarantines rather than from losses in yield or quality (45, 107, 137). Thirty-two countries quickly banned import of U.S. wheat after the discovery of *T. indica* in Arizona in 1996 (92). In the United States, the disease has not spread significantly in the past 10 years and has caused only slight amounts of disease (107), indicating a limited potential for the disease within its current range. Karnal bunt is not present in detectible amounts in some areas where the pathogen can be isolated from field soil (124).

Rice Kernel Smut—*Tilletia horrida*

Rice kernel smut, also known as caryopsis smut, black smut, or grain smut (11), is caused by the pathogen *T. horrida* (**Figure 5**), which, like *T. indica*, causes a partial bunt that affects both yield and quality. *Tilletia horrida* was described in Japan in 1896 (125). By the early 1900s, rice smut was considered widespread in eastern and southern Asia and had been reported in India, Java, Siam, and China (102). Anderson (1) discovered the disease in the United States in 1898 in rice plants from Georgetown, South Carolina, but misidentified the pathogen as *Tilletia corona* Scrib. He reported that the introduction of rice smut into South Carolina was via Japanese rice seed (2). Reyes (102) was the first to observe rice smut in the Philippines in 1920, apparently introduced on rice seed.

Today, rice kernel smut occurs throughout Asia, Australasia and Oceania, in Europe (Greece), Central America (Belize, Cuba, Nicaragua, Panama, and Trinidad), South America (Brazil, Guyana, Surinam, Venezuela), North America (Mexico, United States), and Sierra Leone in Africa (31, 142). A specimen from Senegal, West Africa in Herb. BPI (41) on "*Oryza* sp." suggests the pathogen may be more widespread in Africa than is indicated in current distribution maps. In the United States, rice kernel smut occurs throughout rice-growing regions in Arkansas, California, Louisiana, Mississippi, South Carolina, and Texas (31, 141). In general, it is more severe in Arkansas, Mississippi, and Missouri than in other U.S. rice-growing regions (56).

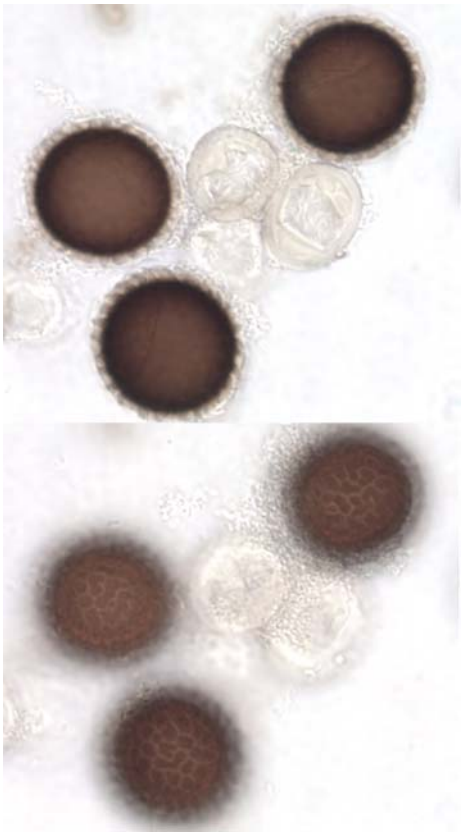

Figure 5

Teliospores of *T. horrida*, cause of rice kernel smut, in median (*top*) and surface (*bottom*) views. Thicker-walled hyaline sterile cells are between the dark teliospores. Teliospores can range from 20 to 38 μm in diameter.

Rice kernel smut is considered a persistent but minor disease throughout rice-growing

Boot stage follows emergence of the flag leaf collar and continues until the spike begins to emerge

regions of the world (11, 26, 91). In general, long-grain rice cultivars, the predominant type grown in the United States, are most susceptible to *T. horrida*, with short-grain rice the least susceptible, and medium-grain rice intermediate in susceptibility (11). Rice containing more than 3% bunted kernels is graded "smutty" (135) and is penalized at the mill (56). Disease incidence as high as 87% and 100% in hybrid rice seed production fields have been reported in Pakistan and China, respectively, although average infection rates in "normal" years are generally less than 25% (11). Even a low incidence of infection can cause substantial economic losses in hybrid rice seed production because a maximum of 0.5% infection is allowed in certified seed in India (11).

MORPHOLOGICALLY SIMILAR BUNT FUNGI OF ECONOMIC IMPORTANCE

Ryegrass Bunt—*Tilletia walkeri*

As a result of regulatory requirements for the import of seed into Arizona, teliospores morphologically similar to *T. indica* (**Figure 6**) were discovered in pasture seed mixes containing wheat and ryegrass from Oregon. This fungus tested positive for *T. indica* using the molecular test in current use at the time (15), although no infected wheat kernels were found. However, differences in color, ornamentation, and size were noted when these teliospores were critically compared with *T. indica*, indicating they represented an undescribed species (24). In early 1997, bunted annual ryegrass seeds (*Lolium multiflorum* L.) were found in the Willamette Valley in Oregon and in wheat samples from the southeastern United States (15). Specimens also were discovered at Washington State University in samples labeled "unidentified *Tilletia* sp." on perennial ryegrass (*Lolium perenne* L.) from Australia's Kangaroo Valley collected in 1967 and 1968 (24). This fungus was later described as the new species,

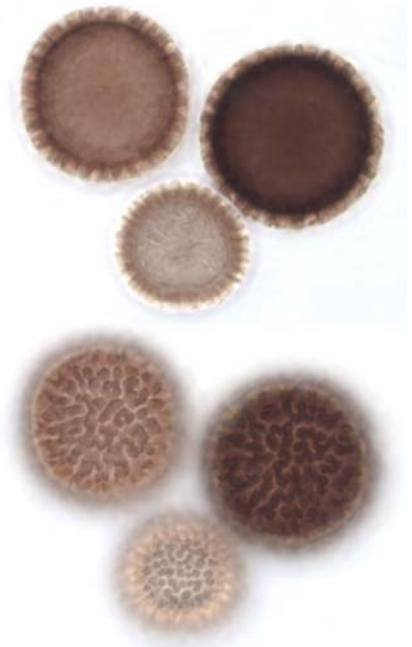

Figure 6

Teliospores of *T. walkeri*, cause of ryegrass bunt, in median (*top*) and surface (*bottom*) views, showing the more coarsely tuberculate to fused and cerebriform ornamentation that distinguishes it from *T. indica*. Teliospores range from 24 to 44 μm in diameter.

T. walkeri Castl. & Carris (24). Its known distribution includes the southeastern United States, Oregon, Australia, and New Zealand (24, 32). Because ryegrass is a common turfgrass species and seed is shipped all over the world, it is expected that the distribution is much more widespread. However, the cryptic nature of the infection (**Figure 7**) necessitates microscopic examination of seed or seed washes to detect it. *Tilletia walkeri* infects *L. multiflorum* and *L. perenne* under natural conditions (24). Artificial inoculation experiments that purportedly showed complete susceptibility of wheat to *T. walkeri* (94) were not reproducible and appear to have resulted from *T. indica* contamination in the original experiments (G. L. Peterson, personal communication). However, boot inoculations of wheat with high concentrations of *T. walkeri* sporidia have rarely resulted in minute sori containing

typical *T. walkeri* teliospores (L. M. Carris & B. J. Goates, unpublished information).

Veldt Grass Bunt—*Tilletia ehrhartae*

The economic impact of Karnal bunt on the United States and Mexican wheat industries spurred other countries to develop diagnostic protocols for distinguishing teliospores of *T. indica* from morphologically similar species (40, 86). In 2004, Australian officials conducted a survey of wheat-exporting facilities to determine the source of *T. indica*-like teliospores that were responsible for rejection of a shipment of Australian wheat by an importing country (93). The teliospores were eventually identified as *Tilletia ehrhartae* Talbot, a systemically infecting tuberculate-spored species (**Figure 8**) that infects Veldt grass (*Ehrharta calycina* Sm.). *Tilletia ehrhartae* has not been reported outside South Africa and Australia.

BIOLOGY OF KARNAL BUNT AND RICE KERNEL SMUT

Teliospore Dormancy

There are three types of teliospore dormancy in *T. indica*. The first is postharvest dormancy. Teliospores taken from freshly harvested grain commonly germinate poorly compared with germination after bunted grains are stored for several months to a year or longer (9, 81, 100, 119, 128). Postharvest dormancy of teliospores also occurs in *T. horrida* (28). Even after teliospores are stored more than one year, germination rarely exceeds 50% under optimal laboratory conditions and is 15%–30% in most reports. This demonstrates the second type of dormancy, which is long-term and likely contributes to teliospore survival under field conditions. The third type of dormancy is induced by cold temperature. In a study of *T. indica*, dry teliospores kept at −18°C progressively lost the ability to germinate over a period of 12 weeks of treatment (146). The teliospores in these experiments were plated immediately after treatment and nongerminability was thought to indicate lethality. However, in subsequent experiments the inhibition of germination was eliminated if teliospores were plated after a

Figure 7

Ryegrass bunt caused by *T. walkeri*. Teliospores appear as small dark dots on the surface of the seed. Sori, structures containing teliospores, are contained within the palea and the lemma and can rarely be observed without microscopic examination of individual seeds.

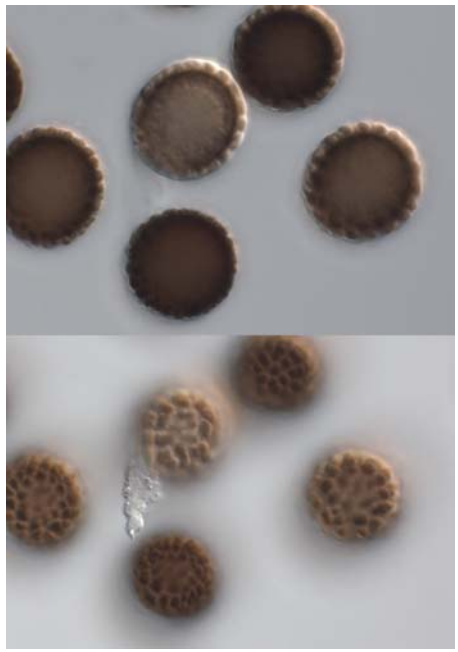

Figure 8

Dark brown teliospores of *T. ehrhartae*, cause of the Veldt grass bunt in Australia and South Africa, in median (*top*) and surface (*bottom*) views. Teliospores of *T. ehrhartae* were found in a survey of Australian wheat consigned for export and range in diameter from 17 to 25 μm.

Retraction septa: secondary or adventitious septa formed in elongating basidium

20-day "thawing" period at 22°C rather than immediately after the cold treatment (27). Cold-induced dormancy has also been observed in experiments performed over 4 to 8 days of freezing at 0°C (111). Similar dormancy also occurs in *Tilletia horrida* (27). Cold-induced dormancy of the dwarf bunt pathogen *T. controversa* Kühn also occurs annually in natural field environments in temperate climates (58, 60).

Teliospore Germination

Most locally infecting bunt fungi that have been studied have echinulate, verrucose, or tuberculate teliospores that germinate at approximately 20°–25°C (25). The effects of physical factors that influence teliospore germination in *T. indica* and *T. horrida* have been studied extensively (28, 35, 72, 76, 119, 125, 127, 146). Germinating teliospores are remarkably durable and resilient during the process even with wide swings in pH, temperature, and soil moisture including freezing and desiccation (35, 119, 144). As in other *Tilletia* species, the basidium (also called a "promycelium") emerges through the ruptured wall of the teliospore and either produces basidiospores immediately, or elongates to over 500 μm in length, depending on the species and the conditions. Elongating basidia form retraction septa, confining the cytoplasm to the apical region (**Figure 9**). A terminal whorl of 10 to 150 primary basidiospores, also called primary sporidia, is formed (**Figure 9**). The number of basidiospores formed per basidium varies considerably among different taxa and not all locally infecting species produce a large number of basidiospores (25). The number of basidiospores can also vary considerably within a species (24, 80, 127). Basidiospores are hyaline, aseptate, filiform to fusiform in shape, ranging from 1.0 to 2.5 μm wide and from 15 to 80 μm in length (L. M. Carris, unpublished information; 95).

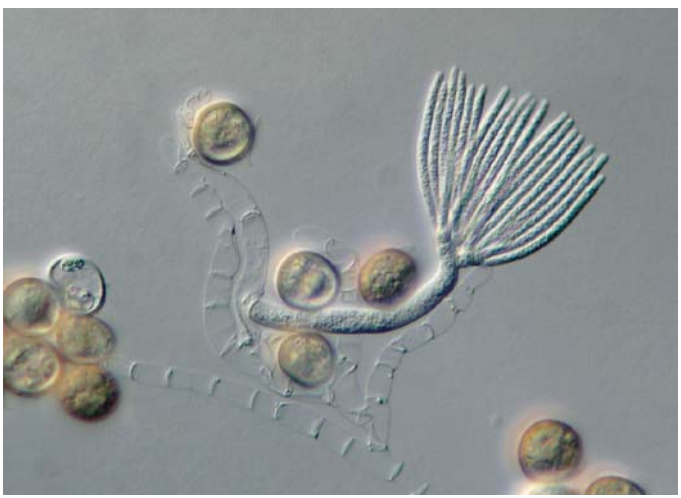

Figure 9

Retraction septa, elongating basidium, and terminal whorl of primary basidiospores in germinating *T. laevis* teliospores. *Tilletia laevis* is a systemically infecting bunt and, along with *T. caries*, causes common bunt of wheat and forms a smaller number of primary basidiospores than *T. indica*, *T. horrida*, *T. walkeri*, and other locally infecting bunt fungi.

Basidiospores may elongate slightly after detachment, and become curved or undulate before germinating to form either hyphae or a sterigma-like structure on which a haploid, uninucleate, allantoid sporidium (**Figure 10**) [also called "ballistospore" (65)] is asymmetrically formed (114). The allantoid sporidia formed by *T. indica*, *T. horrida*, and other locally infecting *Tilletia* species studied have the same morphology and forcible discharge as described for *T. caries* (54). Allantoid sporidia may germinate directly via germ tubes or repetitively to produce additional sporidia (64, 65). In culture, passively dispersed, filiform sporidia (**Figure 11**) are formed from short, lateral sporogenous cells on the hyphae (65). Allantoid sporidia are the primary infective agents of *T. indica* and *T. horrida* but have not been studied in other locally infecting bunts. The role of the passively dispersed, filiform sporidia in the infection process is unclear.

Infection Process

The Karnal bunt disease cycle is initiated when teliospores at or near the soil surface germinate, producing basidiospores that form

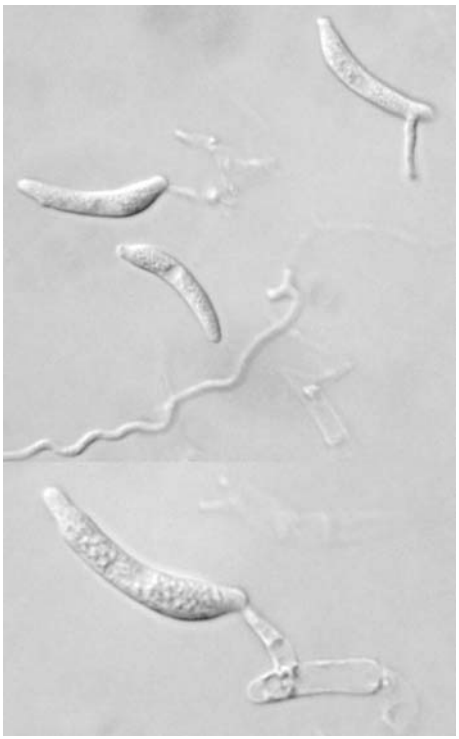

Figure 10

Allantoid secondary sporidia, the primary infective unit of *T. indica* and *T. horrida*.

hyphae, allantoid sporidia, and successive generations of sporidia. The diurnal release of *T. indica* sporidia occurs during periods of high relative humidity primarily between 1800 and 0800 h and is optimal at approximately 0200 to 0300 h (99, 111). Teliospores of *T. horrida* float to the surface of irrigation water in rice fields and germinate after rice is planted (144). Sporidia and hyphae become established in the crop canopy (8) or may form a dense mat on the surface of the rice paddy water in *T. horrida* (126, 141). Some sort of "safety mechanism" has been suggested that insures the presence of ample basidiospores at the time of heading, such as a factor that triggers teliospores to germinate when the plant is heading (138, 144). Otherwise, teliospores would undergo a hypothesized "suicidal germination" (107, 124) if they germinate when the host is not in a susceptible condition.

Initial infection by *T. indica* occurs when sporidia deposited on spikes germinate to produce hyphae that penetrate stomata. Hyphae then grow intercellularly to the base of the floret and enter the periderm of nascent kernels via the funiculus (49). Scanning electron microscopy studies indicate the stomata of the rachis could also be important for initial infection (33). However, information on the movement of hyphae within spikes indicates the rachis is not a regular site for primary infection (101). Nagarajan et al. (88) stated that basidiospores or hyphae of *T. indica* regularly fuse to form a dikaryotic mycelium prior to infection of plants, and basidiospore fusion during culture has been reported (72). During a study of infection in *T. indica*, apparent hyphal anastomosis was only rarely observed prior to penetration of the plant and was not considered a normal means of dikaryon formation (49). Presumably, haploid hyphae are capable of infecting the plant but the dikaryotic state must be attained for teliosporogenesis to occur (39). The stage at which the dikaryon is initiated in *T. indica* is unknown. The method of penetration and timing of dikaryon formation in *T. horrida* are not known, although Singh & Pavgi (116) speculated that sporidia lodge on the feathery stigma and penetrate through the style to the chalazal end of the ovary.

Funiculus: the narrow stalk that attaches the ovary to the base of the flower

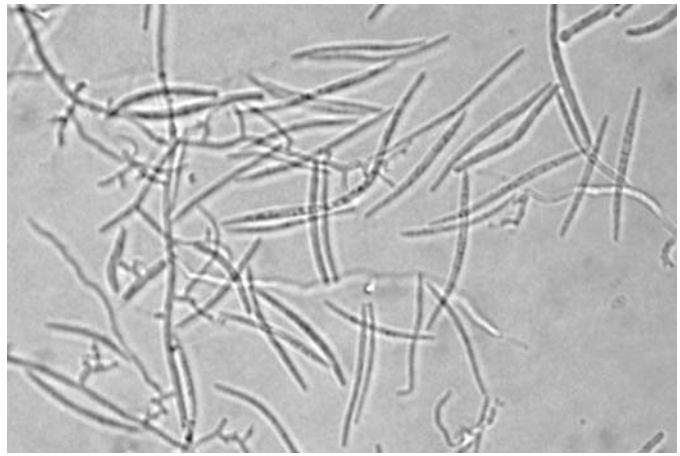

Figure 11

Filiform secondary sporidia, the second type of sporidia produced by species of *Tilletia*.

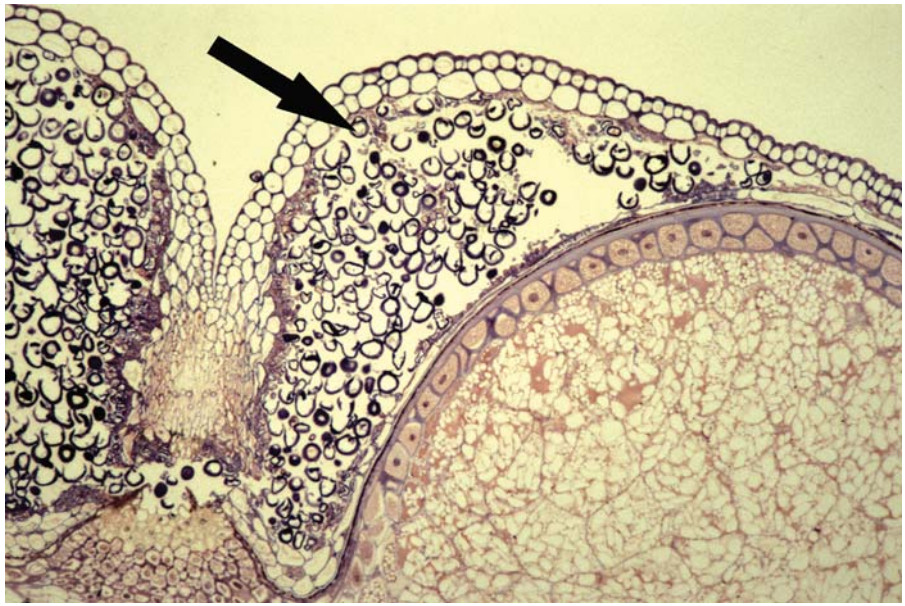

Figure 12

Transversely sectioned wheat kernel infected with *T. indica* showing teliospores (*arrow*) developing within the separating layers of the periderm.

In Karnal bunt and rice kernel smut, disease is manifested by the formation of teliospores in the middle layers of the pericarp (**Figure 12**), which splits and expands as endosperm tissue shrinks (23, 116). The embryo is not colonized and infected seed are often able to germinate, although the embryo is killed in severe infections (47, 116). Teliospores arise from sporogenous cells that form a thin hymenial layer on the surfaces of the cavities formed by the separation of the inner and outer layers of the pericarp and by the separation of the inner pericarp from the seed coat (103). Teliospores of *T. indica* develop at the terminal end of sporogenous hyphae, where the dikaryotic cytoplasm becomes delimited by a septum and karyogamy occurs during enlargement of teliospore initial cells (46, 103). This is essentially the same process observed in systemically infecting *Tilletia* species, except that the teliospore initials form from enlargement of lateral outgrowths or intercalary sections of sporogenous hyphae (96, 132). The process of teliosporogenesis in *T. indica* is influenced by temperature. Even after successful induction of infection, maximum diurnal temperatures of 35°–40°C after the grain-filling stage reduces teliosporogenesis significantly (75).

Epidemiology

Disease spread from season to season occurs when teliospores become dislodged from infected kernels during harvest, become airborne, and settle in the field or spread to adjacent areas with wind currents. Potential long-distance spread of *T. indica* via air is indicated by the presence of viable teliospores at 3000 m above harvested wheat fields where stubble was being burned (14). The Karnal bunt and rice kernel smut pathogens are also spread via infected seed, which presumably was how *T. indica* moved from India into Mexico and subsequently to the United States (78) and how *T. horrida* has moved throughout rice-growing regions of the world.

Teliospores of *T. indica*, *T. horrida*, and other bunt species that have been examined have relatively thick cell walls with four distinct layers—an endosporium, a thin middle layer, an exosporium from which the ornamentation develops, and an outer sheath (57, 89, 96, 103). Reticulate and tuberculate ornamentation in *Tilletia* species are formed

by similar developmental processes (97). The teliospore wall resists toxic gases and liquids including methyl bromide and chloropicrin (120), hydrogen peroxide (123), propionic acid, ozone (107), and degradation in the digestive tract of animals (122). This enables teliospores of *T. indica* to survive for several years in hot desert to cold temperate field environments (6, 16, 17, 29, 72). Under typical laboratory conditions, *T. indica* teliospores can survive for at least 16 years (15), whereas those of *T. horrida* are reported to survive for only 2 to 3 years under laboratory or field conditions (91, 115) and up to 5 months at −18°C (26).

The classic work of Mundkur (84) and Bedi et al. (10) showed that natural infection by *T. indica* occurs via airborne inoculum during heading, but there is considerable disagreement on plant stages that are susceptible, and the most susceptible stage. Results from several studies indicate susceptibility only during specific periods within boot swelling to anthesis stage (3, 7, 13, 15, 48, 68, 74, 87, 107, 109, 112). Studies with *T. indica* aimed at developing reliable and practical methods of inoculation for screening germplasm have demonstrated the highest rate of infection occurs by hypodermically injecting a suspension of sporidia into the wheat boot at awns-emerging stage, or slightly before (3, 4, 7, 30, 105, 106, 117, 139). However, under natural conditions, the spikes are within the boot and airborne inoculum cannot reach the glumes, where hyphal penetration is initiated (49). A recent study with *T. indica* demonstrated that infection from airborne inoculum can occur from the time at which florets begin to emerge from the boot up to the soft dough stage (52). Infection peaked after spikes had completely emerged, but before the onset of anthesis (52). These results conflict with studies claiming susceptibility prior to spike emergence (7, 74, 87, 109), and it is the first report of susceptibility much beyond anthesis. Under certain climatic conditions of heavy dew or light rain, it appears the flag leaf and the boot sheath may be important for natural infection (3, 74). After the initial infection, spread to adjacent florets has been reported to occur as late as the dough stage (34).

The results of numerous studies on teliospore germination demonstrating the wide range of conditions favorable for teliospore germination indicate that teliospore germination per se is not a limiting factor for disease initiation. The production of allantoid sporidia, which are the infective agents of the disease, seems to be the primary factor in epidemiology. Disease-prediction models have been developed that incorporate the climatic factors that influence production of sporidia including temperature, humidity, solar radiation, and rainfall (66, 67, 79). Sporidia have thin cell walls and are considered to be short-lived structures sensitive to desiccation (5, 88, 121). In tests at 95% RH, sporidia of *T. indica* survived no longer than 14 h (121). However, a recent study with *T. indica*, *T. horrida*, *T. walkeri*, and *T. caries* demonstrated that sporidia are remarkably durable. Sporidia were viable for 30 days at 10–20% RH at 20°–22°C and after 60 days at 40%–50% RH at 18°C, and newly formed sporidia were commonly observed germinating within 18 h of rehydration (51). Similar results occurred in field experiments over 46 days at temperatures often exceeding 40°C and relative humidity as low as 10% (B. J. Goates, unpublished information). These results indicate that teliospore germination prior to a susceptible host stage can result in the production of inoculum that remains viable in dry field environments, which then can rapidly regenerate during humid conditions normally associated with disease. Similarly, haploid cells of *Ustilago maydis* appear to survive in soil in the field throughout the year (77), and mycelia of *T. controversa*, dried and then stored at 4°–5°C on filter paper for 7 months, were viable (12).

Dough stage: stage in ripening wheat caryopsis when endosperm develops a mealy or dough-like consistency

Flag leaf: the last leaf formed on a developing wheat plant

Mating and Nuclear Behavior

Nuclear behavior of *T. indica* during teliospore germination was found to be the same as in

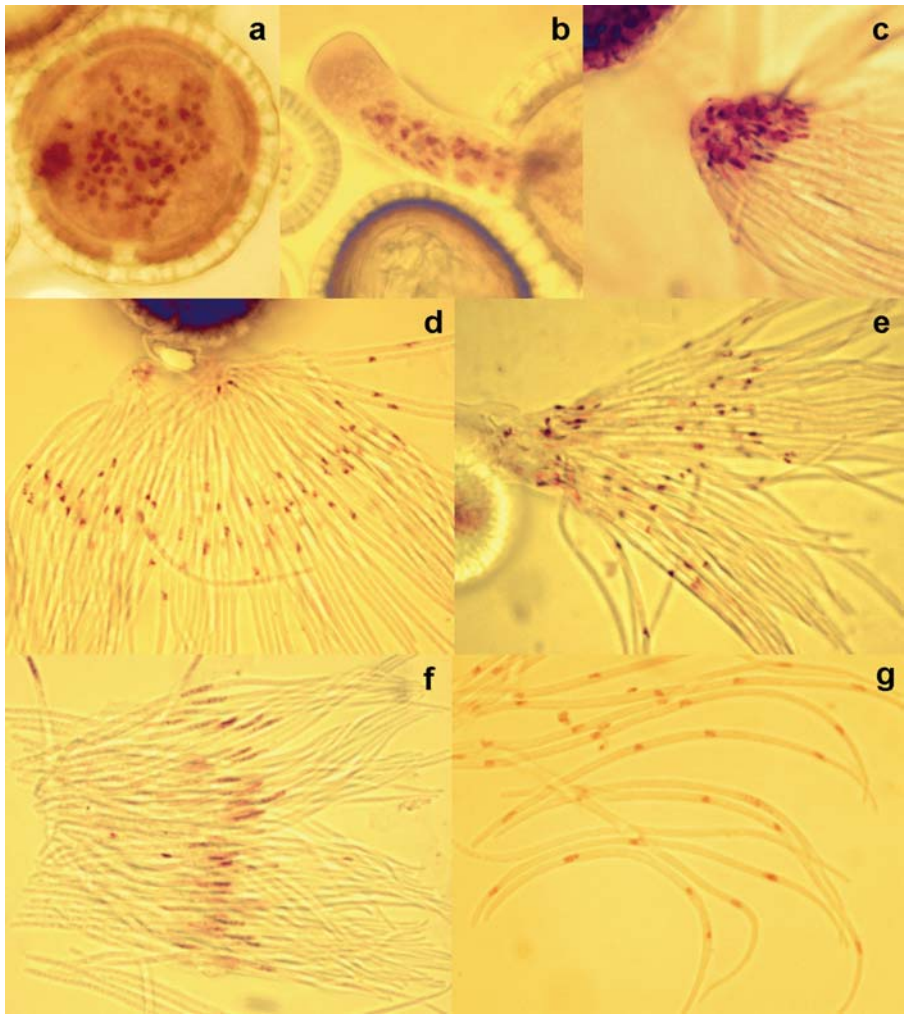

Figure 13

Nuclear condition of teliospores and basidiospores prior to, during, and following the germination process. (*a*) Numerous haploid nuclei in a *T. indica* teliospore just prior to germination. (*b*) Nuclei migrating into the elongating basidium (promycelium). (*c*) Nuclei tightly grouped near the tip of the basidium migrating into the basidiospores (primary sporidia). (*d*) Nuclei in basidiospores just prior to mitosis. (*e*) Nuclei just after mitosis. One of each daughter nucleus is in the process of migrating back into the basidium. (*f*) Single interphase nuclei in each basidiospore after detachment from the basidium. (*g*) Multinucleate basidiospores resulting from mitotic divisions of the haploid nuclei.

common and dwarf bunt fungi (55). During the early stages of teliospore germination, the diploid nucleus undergoes meiosis followed by several rounds of mitosis, producing numerous haploid nuclei (**Figure 13*a***) prior to the formation of the basidium (55). Singh & Pavgi (114) showed in *T. horrida* that meiosis occurred in the teliospore prior to basidium formation, but reported the subsequent mitotic divisions occurred in the basidium. In *T. indica*, approximately 64 to 128 nuclei were observed migrating into the basidium (**Figure13*b***) (B. J. Goates, unpublished information). Nuclei migrate into developing

basidiospores, divide synchronously, and one daughter nucleus returns to the basidium. These nuclei in the basidium then either enter empty basidiospores or degenerate (13, 55). The sorting of 64 or more nuclei that are tightly grouped at the tip of the basidium (**Figure 13*c***) followed by orderly migration of 1 nucleus into each of approximately 100 basidiospores is a remarkable feat of cellular mechanics. Static images of this process and subsequent mitosis (**Figures13*d–f***) appear chaotic, but the result is uninucleate basidiospores (**Figure 13*f***) incapable of producing disease alone. The production of exclusively haploid basidiospores is demonstrated by the lack of pathogenicity in single basidiospore lines in both *T. indica* and *T. horrida* (39, 144). Mitotic divisions of single haploid nuclei and subsequent formation of septa in the basidiospores after the formation of a basal septum to delimit the basidiospore result in two- to four-celled basidiospores (**Figure 13*g***) (46). Each haploid cell of the basidiospore can produce hyphae, or form allantoid or filiform sporidia.

Tilletia indica is heterothallic and bipolar with four alleles controlling mating and pathogenicity (39, 43). Erroneous reports that *T. horrida* is homothallic (91) are based on the presence of two nuclei in detached basidiospores and the lack of conjugation between basidiospores (114). However, inoculation studies using single and paired monospore lines of *T. horrida* clearly demonstrated the pathogen's heterothallic nature (144). The mating systems of other locally infecting species of *Tilletia* have not been studied. In systemically infecting species, a bipolar, multiallelic mating system is present in *T. controversa* (59), and a bipolar, biallelic system is present in *T. caries* and *T. laevis* Kühn (61–63).

Visualization of chromosomes in bunt fungi by traditional staining techniques used in higher organisms and some fungi has been impossible due to the small size and compact nature of nuclei during mitosis and meiosis (53, 55). Based on electrophoretic karyotyping, the number of chromosomes in *T. indica* was found to vary from 11 to 13 (130). Similar variation also occurs in *T. controversa* and *T. caries* (108). The variability is attributed to chromosome-length polymorphisms and polyploidy in conjunction with aneuploidy. Variable numbers of chromosomes can be found among monospore isolates from single teliospores of *T. indica*, indicating that chromosomal alteration or differential segregation occurs during meiosis (130).

Bipolar mating system: incompatibility system with one locus that has two (biallelic) or multiple (multiallelic) alleles

Host Ranges

Host range lists for *Tilletia* species contain naturally occurring hosts, hosts that have been infected via artificial inoculation (106, 140), and hosts resulting from synonymy of similar species (37, 38). Artificial inoculation methods used for the locally infecting smuts include deposition of inoculum onto spikes, introduction of sporidia into the developing floret, and injection of sporidia into the plant boot stage using a hypodermic syringe (10, 30, 39, 49, 52, 105, 106, 140, 144). The latter method is most reliable and has become the method of choice for host-range studies (105, 106) and field screening (3, 47, 140), but it is also highly artificial and presumed to measure physiological rather than field resistance (140).

Tilletia indica infects bread wheat (*Triticum aestivum* L.), durum wheat (*T. turgidum* Desf.), and triticale (X *Triticosecale* Wittm.) under natural conditions (47). The wild wheat species *Aegilops geniculata* Roth (as *T. ovatum*), A. *sharonensis* Eig., *A. peregrina* (Hack.) Maire & Weiller var. *peregrina* (as *T. variabilis*), and "*Triticum scerrit*" are reported as hosts for *T. indica*, but it was not indicated if they occurred naturally (5). With artificial inoculation, *T. indica* infected triticale, 3 species of *Triticum* L., 11 species of *Aegilops* L., 2 species of *Bromus* L., 3 species of *Lolium* L., and *Oryzopsis miliacea* (L.) Benth. & Hook (106).

Under natural conditions, *T. horrida* has been reported only from rice (*Oryza* L.), but erroneous reports in the literature have led to considerable confusion over its host range and biology. Early reports of rice smut in the

southern United States, including Louisiana in 1903 and Arkansas in 1926, must be interpreted in light of the original misidentification of the pathogen as *T. corona*, which at the time was reported to occur on *Digitaria* Haller, *Leersia* Sw., and *Panicum* L. (131). Tullis & Johnson (134) synonymized *T. horrida* with *Tilletia barclayana* (Bref.) Sacc. & Syd. based on questionable results of a study in which seedlings of two species of *Pennisetum* L.C. Rich. were artificially infected with the rice pathogen. Although the rice plants were not infected and the resulting teliospores from the inoculated species of *Pennisetum* were morphologically more similar to *T. barclayana* than to *T. horrida*, this was considered proof of synonymy (134). In tests of 32 species of grasses, *T. horrida* infected only rice and, surprisingly, the wheat relative *Aegilops sharonensis* (106). Whitney (143) showed in other tests that *T. horrida* infected rice, but not *Pennisetum*, and that seedling inoculations did not result in infection. However, the synonymy of *T. horrida* with *T. barclayana* prevailed until molecular data proved the species were genetically distinct (25, 98).

EVOLUTION OF TILLETIALES

Approximately 140 species are currently recognized in the genus *Tilletia*, all of which infect hosts in the grass family Poaceae. Most of these have not been subjected to critical study, including the mode of infection. However, nearly half of the 42 species included in a phylogenetic study of the Tilletiales were locally infecting, based on the occurrence of only a few partially diseased ovaries per inflorescence (25). These locally infecting species consisted of a genetically diverse group, most of which occur on hosts outside the grass subfamily Pooideae. Most of the systemically infecting species that have been studied occur on hosts in the Pooideae and have reticulate teliospores that germinate to form less than 20 rapidly conjugating basidiospores, whereas most locally infecting species have tuberculate to verrucose teliospores that germinate to form nonconjugating basidiospores. The locally infecting character is found in the more basal lineages and is likely the ancestral character in the group. *Tilletia indica* and *T. walkeri*, also on pooid hosts, may be exceptional species in that they are more closely related to the reticulate-spored, systemically infecting species of *Tilletia* than to other tuberculate-spored, locally infecting species (25). The similar type of teliospore morphology, germination, and infection type shared by *T. indica* and *T. horrida* have been considered indicative of a close relationship, but we now know that these species are only distantly related.

CONCLUSIONS/SUMMARY

The genus *Tilletia* comprises a monophyletic group of species that have teliospores with smooth, reticulate, or tuberculate ornamentation, local or systemic infection, and conjugating or nonconjugating basidiospores (25, 136). Relatively few *Tilletia* species infect economically important hosts, but even smut fungi on noncultivated hosts such as *T. ehrhartae* and *T. walkeri* can become economically important and cause trade restrictions when they occur as contaminants in grain and seed, where accurate identification is often not possible.

In the systemically infecting species, the dikaryon is formed by the rapid conjugation of compatible basidiospores (the characteristic "H-body"), and infection occurs at the host seedling stage by dikaryotic infection hyphae originating from the conjugated basidiospores or sporidia (50). In the locally infecting smuts, the basidiospores germinate to form monokaryotic hyphae or sporidia. However, the precise time and location of dikaryon infection in the locally infecting species remains unknown. Dikaryon formation in *Ustilago* (Pers.) Roussel, another genus of smut fungi, involves the production of pheromones under the control of the mating locus [summarized in (73)] and may be similarly controlled in *Tilletia*. Unfortunately, characterization of mating loci in *Tilletia* species lags

behind that of *Ustilago*, in part because of the greater difficulty in manipulating *Tilletia* species in culture. Many of the locally infecting species grow vigorously in culture (L. M. Carris, unpublished information), potentially enabling manipulation, characterization, and further study to advance our understanding of the biology and genetics in the genus *Tilletia*.

SUMMARY POINTS

1. The locally infecting smuts *Tilletia indica* and *T. horrida* generally cause minor losses in yield and quality in countries where they occur. Significant economic impact occurs due to the effects of international quarantines for Karnal bunt and low tolerance levels in certified hybrid rice seed for rice kernel smut.
2. Forcibly discharged sporidia play a critical role in the epidemiology of locally infecting smuts. Infection occurs via sporidia that are deposited on the spikes of susceptible host plants.
3. Recent studies show that sporidia are remarkably durable, able to survive extensive periods of low humidity in laboratory and field conditions. The timing of teliospore germination may not be critical in the locally infecting smuts as the sporidia remain viable under dry conditions and regenerate under favorable conditions.
4. The process of dikaryon formation is poorly understood in the locally infecting smuts. With the exception of *T. indica*, the infection process is poorly documented in the locally infecting smuts.
5. Phylogenetic analysis of the Tilletiales demonstrates that the genus *Tilletia* encompasses both locally and systemically infecting species with teliospores that germinate to form a variable number of conjugating or nonconjugating basidiospores. There is no support for the recognition of *Neovossia* as distinct from *Tilletia*.

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